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Antibacterial and antifungal activity of *Mentha longifolia* Mirkalan village of Nizampur region Nowshehra district of KPK, Pakistan

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Abstract

Mentha longifolia Mirkalan village of Nizampur region Nowshehra district of KPK, Pakistan leaves were collected and air dried for seven days and crushed into powder form. The extracts were prepared in 70 percent ethanol and hot water and preserved for susceptibility testing. The selected microorganisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans* and *Aspergillus niger* were cultured on nutrient agar through spreading technique by using a swab. After culturing the wells were made on nutrient agar by cork borer of 6mm in diameter. The wells were then filled with prepared extracts and also control was applied for valid results. Then the plates were incubated for overnight and next day the clear zones of inhibition around the wells were measured in mm. Ethanolic extract of *Mentha longifolia* shows the high zone of inhibition as compared to the hot water extract. The highest zone of inhibition by Ethanolic extract is 29mm against *Enterococcus faecalis* and 27mm against *Escherichia coli*. 25mm and 20mm of clear zones were appeared against fungi named as *Candida albicans* and *Aspergillus niger* respectively. While hot water extracts show highest zone of inhibition was against *E. coli* and *Candida albicans* which was 15mm and 10mm respectively.

Keywords: Antibacterial, antifungal activity, *Mentha longifolia*, Mirkalan village

Introduction

Mentha longifolia belongs to family Lamiaceae and genus *Mentha* has 5 groups which are *Audibertia*, *Pulegium*, *Preslia*, *Mentha* and *Eriodontes* [1, 2]. On the basis of differences in inflorescence from the *Mentha* group is further divided into 3 subgroups, which are Capitatae, Verticillatae and Spicatae [2, 3]. The species are still not defined properly, but comprise of more than 25 species in the *Mentha* genus which grows all over the world [4]. Some of the species belonging to the *Mentha* genus which are known for its peppermint, menthol, mint, spearmint and wild mint are cultivated in Europe, East Asia, Australia, and America. These species which are cultivated due to its characteristics are *Mentha piperita*, *Mentha arvensis*, *Mentha picata* and *Mentha longifolia* [4, 5]. The species *Mentha longifolia* is also called as a wild mint. This specie is a perennial herb which can grow at about 1 to 2 meters long and is one of the most fast growing specie in all species of this genus. The stem of the *Mentha longifolia* is square in shape, having left opposite to each other in pairs [6]. These leaves smell like lemon or menthol [7]. The oil extracted from *Mentha longifolia* shows activity as anti-oxidative, cytotoxic and antimicrobial [8, 9]. Mostly this species is used as an herbal remedy for the relief from colds, cough, stomach cramps, flatulence, asthma, headaches and disturbance in digestion. Also used as an ointment in treatment of wounds [10, 11]. Leaves of *Mentha longifolia* can also be used in salad and cooked food for flavoring purposes [12]. The species belongs to the genus *Mentha* are mostly reported from several studies of having antibacterial activity, antifungal activity [13-16], and insecticidal activity [17]. As bacterial and fungal resistance evolving, so many drugs is one of the major problems of worldwide due to which the world is facing difficulties in the treatment of several infections [18]. Due to the evolving of resistance by bacteria to the present drugs, the medicinal plants such as *Mentha longifolia* are gaining the importance for development of new drugs against these resistive drugs [19-21]. Drugs developed from these medicinal plants also are more specific, simple in structure and have negligible side

effects on body [22].

Domestically leaves of *Mentha longifolia* is used in tea for the relief in different conditions such as headache, fevers, bronchitis, ulcerative colitis, disorders of liver and other minor diseases [23-25].

As previous studies show that *Mentha longifolia* is used traditionally for the treatment of several complications. So, the study was conducted to evaluate the antibacterial and antifungal activity of leaf extracts of *Mentha longifolia* against some strains of bacteria and fungi.

Materials and Methods

Mentha longifolia was collected from the Mirkalan village of Nizampur region Nowshehra district of KPK, Pakistan in first week of November 2016 and brought to Abbottabad University of Science and Technology Havelian, where Doctor Mujaddad Ur Rehman identified the plant at the Microbiology department Lab. The leaves were collected and air dried at room temperature by placing in the laboratory for 7 days. After drying of leaves at room temperature, the dried leaves were then pulverized into powder. The powder form of leaves was then stored in the glass container with a tight cap to avoid the contamination in powdered leaves of *Mentha longifolia* until the extract was not prepared [26]. The extracts of leaves were then prepared in different chemicals such as ethanol 70 percent and hot water. 20g of powder were soaked in 120ml ethanol while 20g of powder were soaked in 100 ml hot water and were placed separately on shaker for at least 1 day. After dissolving on shaker each of extract were filtered through Whatman no. 1 filter paper. For obtaining the dried extract for further use in future the filtrate were placed in a rotary evaporator. After obtaining the dried extract, it was stored in air tight screw capped tube at 40 °C for further use [27].

Well Diffusion Method

For the analysis of the susceptibility of leaf extract of *Mentha longifolia*, the agar well diffusion method was applied. In well diffusion method first step was to inoculate the selected strains of fungi and bacteria on the nutrient agar plates. The inoculum was taken from the selected colonies of microbes through swab and spread all over the nutrient agar. After spreading method the wells were made on agar media through cork borer, which was about 6mm in diameter. After making of wells, these wells were then filled by prepared leaves extract of *Mentha longifolia* and placed an incubator for overnight at 37 °C for bacterial plates and 25 °C for fungal containing plates. Both gram positive and gram negative species of bacteria were selected for this study such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* while the selected fungal strains were *Candida albicans* and *Aspergillus niger* [28].

Methanolic extract of *Mentha longifolia* was used in the concentration of about 300µg/well while extract of leaves in hot water was used in concentration of 200µg/well. After incubation of plates containing these extracts in wells, the next day clear zones of inhibition were measured which shows the activity of leaf extracts of *Mentha longifolia* against that type of fungi and bacteria.

Results

The leaf extracts of *Mentha longifolia* obtained from ethanol and hot water were applied on different selected strains of bacteria and fungi which were *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Candida*

albicans and *Aspergillus niger*. All of these selected microorganisms were cultured on nutrient agar and wells of 6mm in diameter were made after culturing. The extracts were poured in these wells separately and incubated for overnight and zone of inhibition were measured in mm after incubation. Erythromycin and Ampotericin were also applied to the plates as a control for bacteria and fungi respectively. Ethanolic extract of *M. longifolia* shows the highest zone of inhibition against *Enterococcus faecalis* which was about 29 mm and *E. Coli* which was 27 mm. It also shows activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which was 23mm and 12mm respectively (Table1). A lowest zone of inhibition appeared against *Pseudomonas aeruginosa* after incubation. Ethanolic extract also shows good activity against selected fungi which was 25mm against *Candida albicans* and 20mm against *Aspergillus niger* (Table3).

Table 1: Zone of inhibition of Ethanolic leaf extract of *M. Longifolia* against bacteria.

Bacteria	Ethanolic zone of inhibition of <i>M. Longifolia</i>	Erythromycin zone of inhibition
<i>E. Coli</i>	27mm	20mm
<i>Staphylococcus aureus</i>	23mm	18mm
<i>Pseudomonas aeruginosa</i>	12mm	10mm
<i>Enterococcus faecalis</i>	29mm	23mm

The extract in hot water when applied on selected microorganism also shows some activity but not more than ethanolic extracts. The zone of inhibition appeared due to extract in hot water was 15mm against *E. Coli*, 14mm against *Staphylococcus aureus*, 8mm against *Pseudomonas aeruginosa* and 14 mm against *Enterococcus faecalis*. The highest zone of inhibition was observed against *E. coli* out of all selected bacteria which was 15mm in diameter (Table2).

Table 2: Zone of inhibition of Hot water leaf extract of *M. Longifolia* against bacteria.

Bacteria	Hot water zone of inhibition of <i>M. Longifolia</i>
<i>E. Coli</i>	15mm
<i>Staphylococcus aureus</i>	14mm
<i>Pseudomonas aeruginosa</i>	8mm
<i>Enterococcus faecalis</i>	14mm

The clear zones appeared due to the hot water extract of *M. longifolia* against fungi were 10mm against *Candida albicans* while 7mm against *Aspergillus niger* (Table3).

Table 3: Zone of inhibition of extracts of *M. Longifolia* against fungi.

Fungi	Ethanolic zone of inhibition of <i>M. longifolia</i>	Hot water zone of inhibition of <i>M. longifolia</i>	Ampotericin zone of inhibition
<i>Candida albicans</i>	25mm	10mm	23mm
<i>Aspergillus niger</i>	20mm	7mm	20mm

Discussion

Plants are one of the natural sources having medicinal activity

which needed to be searched for novel medicines that can be used against microorganisms. *Mentha longifolia* is one of the medicinal plants having antimicrobial activity of many microorganisms, including gram positive and gram negative bacteria also have activity against fungi. This study was carried out for such purposes to investigate the activity of leaf extracts of *Mentha longifolia*. Leaves of *Mentha longifolia* were collected and dried which was then grinded to powder form and their extracts were prepared in 70 percent ethanol and also in hot water. Selected bacteria in this study were *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. While the selected fungi were *Candida albicans* and *Aspergillus niger*.

Nutrient agar was used for performing the susceptibility test. Selected microorganisms were first inoculated and spread on nutrient agar and then wells of 6mm diameter through cork borer were made on each plate. The prepared extracts were poured in these wells and also control was applied for most valid results which were Erythromycin and Ampotericin for bacteria and fungi respectively. These cultured plates along with the extract and control were incubated for overnight and next day the clear zones of inhibition were measured in mm. The results of this study show the highest zone of inhibition for Ethanolic extract as compared to hot water which is similar to reported by Cushnie [29]. The reason of highest zone of inhibition due to Ethanolic extract is due to the presence of flavonoids which indicates that *M. longifolia* can be used in pharmaceutical industries [30]. A zone of inhibitions in this study observed against selected bacteria are 27 mm, 23mm, 12mm and 29mm to *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* respectively. The results obtained in this study were similar to another study such as Stanisavljević *et al* [31]. A zone of inhibition to fungi in this study was 25mm against *Candida albicans* and 20mm to *Aspergillus niger*.

Extracts in hot water of *Mentha longifolia* shows low activity as compared to the Ethanolic extracts which were 15mm, 14mm, 8mm, 14mm, 10mm and 7mm against *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Candida albicans* and *Aspergillus niger* respectively. The present study shows the best activity of *Mentha longifolia* against *E. coli* and *Staphylococcus aureus* which is same as compared to the study of Hafedh *et al* [32] but this study also included the *Enterococcus faecalis* showing 29mm of clear zone due to an ethanolic extract of *Mentha longifolia*. Other studies also reported the antimicrobial activity of *Mentha longifolia* against *E. coli*, *P. aeruginosa* and *Staph aureus* [33, 34]. More studies are needed on *Mentha longifolia* for evaluation of its activity such as against resistant strains of microorganisms which is one of the major problems of all over the world.

Conclusion

The present study concluded that an ethanolic extract of *Mentha longifolia* has the best activity against *Enterococcus faecalis*, *Staph aureus* and *E. coli* as compared to the hot water extract. This plant also shows activity against fungi such as *Candida albicans* and *Aspergillus niger*. So this plant can be used in pharmaceutical industries and for evaluating novel medicines against many bacteria and fungi which can be helpful in better treatment in future with negligible side effects on the body.

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